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1. Document ID: US 5939391 A

Entry 1 of 4 File: USPT Aug 17, 1999

US-PAT-NO: 5939391
DOCUMENT-IDENTIFIER: US 5939391 A

TITLE: Hemoglobin alpha chain peptide fragments useful for inhibiting stem cell proliferation

DATE-ISSUED: August 17, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsyrlova; Irena	Gaithersburg	MD	N/A	N/A
Wolpe; Stephen D.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 514/14; 530/326, 530/327, 530/385

ABSTRACT:

Disclosed and claimed are methods for the isolation and use of stem cell inhibiting factors for regulating the abnormal stem cell cycle and for accelerating the post-chemotherapy peripheral blood cell recovery. Also disclosed and claimed are the inhibitors of stem cell proliferation.
7 Claims, 28 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

2. Document ID: US 5861483 A

Entry 2 of 4 File: USPT Jan 19, 1999

US-PAT-NO: 5861483
DOCUMENT-IDENTIFIER: US 5861483 A

TITLE: Inhibitor of stem cell proliferation and uses thereof

DATE-ISSUED: January 19, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wolpe; Stephen D.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 530/385; 435/69.1, 530/350, 530/380

ABSTRACT:

The present invention provides polypeptides and compositions containing same which include a hemoglobin alpha chain wherein the C-terminal hydrophobic domain has been modified.
15 Claims, 31 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 27

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

3. Document ID: CN 1220670 A, WO 9736922 A1, AU 9724391 A, NO 9804628 A, EP 891377 A1, US 5861483 A

Entry 3 of 4 File: DWPI Jun 23, 1999

DERWENT-ACC-NO: 1997-503044
DERWENT-WEEK: 199943

TITLE: Haemoglobin alpha chain derivatives - used to inhibit or stimulate stem cell proliferation, for treatment of cancer, immune depression etc
INVENTOR: TSYRLOVA, I; WOLPE, S D

PRIORITY-DATA:
1996US-0627173 April 3, 1996

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
CN 1220670 A	June 23, 1999	N/A	000	C07K014/00
WO 9736922 A1	October 9, 1997	E	162	C07K014/00
AU 9724391 A	October 22, 1997	N/A	000	C07K014/00
NO 9804628 A	December 1, 1998	N/A	000	C07K014/805
EP 891377 A1	January 20, 1999	E	000	C07K014/00
US 5861483 A	January 19, 1999	N/A	000	C07K005/00

INT-CL (IPC): A61 K 38/19; A61 K 38/42; C07 K 5/00; C07 K 14/00; C07 K 14/805

ABSTRACTED-PUB-NO: US 5861483A
BASIC-ABSTRACT:

[illegible] therapy [illegible], but more generally whenever a mammal is exposed to an agent, [illegible] or survival, that damages or destroys SCs. It can also be used to stimulate growth of B cells, and to treat myeloproliferative, autoimmune or epithelial stem cell [illegible]

conjunction with a cytokine (I) can be used for ex vivo expansion of SCs. (All claimed). INPROL and opiates are used similarly, but can also be used for control of SC hypoproliferation (e.g. aplastic anaemia), for treating or preventing SC exhaustion (e.g. where caused by acquired immune deficiency syndrome), and to treat or prevent immunodeficiency. INPROL can also be used for treating pain in a mammal. (All claimed). Haematopoietic cells can be treated with (I) ex vivo, either for subsequent return to a patient (after myeloablative therapy for leukaemia etc.) or for ex vivo maintenance.

ADVANTAGE - INPROL and related compounds reversibly inhibit or stimulate SC depending on the dose, so allow precise control over cycling of these cells.
ABSTRACTED-PUB-NO:

WO 9736922A EQUIVALENT-ABSTRACTS:

A polypeptide (I) comprising the haemoglobin (Hb) alpha chain in which the C-terminal hydrophobic domain or haptoglobin-binding domain has been deleted or substituted, is new.

USE - (I) is used to inhibit SC proliferation, particularly during radiotherapy or chemotherapy of cancer, but more generally wherever a mammal is exposed to an agent, e.g. an antiviral, that damages or destroys SCs. (I) can also be used to stimulate growth of B cells, and to treat myeloproliferative, autoimmune or epithelial stem cell hyperproliferation (e.g. myelodysplastic syndrome). It can also be used for differential protection of normal SC, but not cancer cells, from chemotherapy or radiation, particularly after normal SC have been induced to proliferate by therapeutic treatment. (I) is used as an adjuvant before, during or after vaccination, to treat immunodepression caused by SC hyperproliferation, to treat ex vivo transfected haematopoietic cells before use in gene therapy (optionally followed by in vivo treatment with (I)). When used in conjunction with a cytokine (I) can be used for ex vivo expansion of SCs. (All claimed). INPROL and opiates are used similarly, but can also be used for control of SC hypoproliferation (e.g. aplastic anaemia), for treating or preventing SC exhaustion (e.g. where caused by acquired immune deficiency syndrome), and to treat or prevent immunodeficiency. INPROL can also be used for treating pain in a mammal. (All claimed). Haematopoietic cells can be treated with (I) ex vivo, either for subsequent return to a patient (after myeloablative therapy for leukaemia etc.) or for ex vivo maintenance.

ADVANTAGE - INPROL and related compounds reversibly inhibit or stimulate SC depending on the dose, so allow precise control over cycling of these cells.

4. Document ID: US 5939391 A, WO 9610634 A1, AU 9537257 A, NO 9701444 A, FI 9701000 A, EP 784677 A1, MX 9702266 A1, ZA 9508253 A, JP 10509422 W, NZ 294446 A

DERWENT-ACC-NO: 1996-209356
DERWENT-WEEK: 199939

TITLE: Inhibitors of stem cell proliferation comprising haemoglobin chains - useful in mammals, e.g. for protecting stem cells from antiviral agents, treating cancer, and maintaining mammalian haematopoietic stem cells ex vivo
INVENTOR: KOZLOV, V; TSYRLOVA, I ; WOLPE, S D

PRIORITY-DATA:

1995US-0535882	September 28, 1995
1994US-0316424	September 30, 1994

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5939391 A	August 17, 1999	N/A	000	A61K037/00
WO 9610634 A1	April 11, 1996	E	101	C12N015/00
AU 9537257 A	April 26, 1996	N/A	000	C12N015/00
NO 9701444 A	May 26, 1997	N/A	000	A61K000/00
FI 9701000 A	May 28, 1997	N/A	000	C12N000/00
EP 784677 A1	July 23, 1997	E	000	C12N015/00
MX 9702266 A1	June 1, 1997	N/A	000	C12N015/00
ZA 9508253 A	June 24, 1998	N/A	100	A61K000/00
JP 10509422 W	September 14, 1998	N/A	093	A61K038/16
NZ 294446 A	July 29, 1999	N/A	000	C07K007/06

INT-CL (IPC): A61 K 0/00; A61 K 37/00; A61 K 38/00; A61 K 38/16; A61 K 38/18; A61 K 38/19; A61 K 38/20; A61 K 38/21; A61 K 38/22; A61 K 38/42; A61 K 39/00; A61 K 45/05; A61 K 48/00; A61 N 5/00; C07 K 1/00; C07 K 5/ 087; C07 K 5/107; C07 K 7/06; C07 K 7/08; C07 K 7/50; C07 K 14/00; C07 K 14/805; C12 N 0/00; C12 N 5/00; C12 N 5/10; C12 N 15/00; C12 N 15/09; C12 N 15/12

ABSTRACTED-PUB-NO: US 5939391A
BASIC-ABSTRACT:

A novel pharmaceutical compsn. comprises a polypeptide selected from the haemoglobin (Hb) alpha, beta, gamma, delta, epsilon or zeta-chains and a carrier.

USE - The compsn. is an inhibitor of stem cell proliferation (INPROL), and is useful for this purpose, partic. in mammals exposed to an agent, e.g. an antiviral agent, which damages or destroys stem cells (claimed). It is also useful for stimulating the growth of B cells (claimed). INPROL can also be used with ratio- or chemotherapy to treat cancer in mammals by differentially protecting normal stem cells and not cancer cells from such therapy. (claimed). INPROL is also useful for maintaining mammalian haematopoietic stem cells ex vivo, partic. bone marrow, (mobilised) peripheral blood or cord blood cells (claimed). It is also useful for ex vivo cell expansion of haematopoietic cells in combination with 1 stimulatory cytokine (claimed). INPROL can also be used to treat myeloproliferative or autoimmune disease, partic. a myelodysplastic syndrome, or epithelial stem cell hyperproliferation in mammals (claimed).

ADVANTAGE - The novel INPROL peptides are different from those already known. They have mol. wt. in excess of 10000 daltons. They are more hydrophobic than the MIP-1alpha or TGFbeta, and their mode of action is different in that they are active in an in vitro assay when used during a preincubation period only.

ABSTRACTED-PUB-NO:

WO 9610634A EQUIVALENT-ABSTRACTS:

A novel pharmaceutical compsn. comprises a polypeptide selected from the haemoglobin (Hb) alpha , beta , gamma , delta , epsilon or zeta-chains and a carrier.

USE - The compsn. is an inhibitor of stem cell proliferation (INPROL), and is useful for this purpose, partic. in mammals exposed to an agent, e.g. an antiviral agent, which damages or destroys stem cells (claimed). It is also useful for stimulating the growth of B cells (claimed). INPROL can also be used with ratio- or chemotherapy to treat cancer in mammals by differentially protecting normal stem cells and not cancer cells from such therapy. (claimed). INPROL is also useful for maintaining mammalian haematopoietic stem cells ex vivo, partic. bone marrow, (mobilised) peripheral blood or cord blood cells (claimed). It is also useful for ex vivo cell expansion of haematopoietic cells in combination with at least 1 stimulatory cytokine (claimed). INPROL can also be used to

[illegible]

TGFbeta, and their mode of action is different in that they are active in an in vitro assay when used during a preincubation period only.

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

Term	Documents
INPROL	4
INPROLS	0
INPROL	4

Display 25 Documents including document number 4

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Document Number 1

Entry 1 of 4 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939391 A
TITLE: Hemoglobin alpha chain peptide fragments useful for inhibiting stem cell proliferation

BSPR:
The present invention relates to polypeptides which are inhibitors of stem cell proliferation ("INPROL") and their use.

BSPR:
The present invention also comprises pharmaceutical compositions containing INPROL for treatment of a variety of disorders.

BSPR:
In another aspect, the invention provides a method for protecting and restoring the hematopoietic, immune or other stem cell systems of a patient undergoing chemotherapy, which includes administering to the patient an effective amount of INPROL.

BSPR:
In still a further aspect, the present invention involves a method for adjunctively treating any cancer, including those characterized by solid tumors, by administering to a patient having cancer an effective amount of INPROL to protect stem cells of the bone marrow, gastrointestinal tract or other organs from the toxic effects of chemotherapy or radiation therapy.

BSPR:
Yet another aspect of the present invention involves the treatment of leukemia, comprising treating hematopoietic cells having proliferating leukemia cells therein with an effective amount of INPROL to inhibit proliferation of normal stem cells, and treating the bone marrow with a cytotoxic agent to destroy leukemia cells. This method may be enhanced by the follow-up treatment of the bone marrow with other agents that stimulate its proliferation; e.g., colony stimulating factors. In one embodiment this method is performed in vivo. Alternatively, this method is also useful for ex vivo purging and expansion of hematopoietic cells for transplantation.

BSPR:
In still a further aspect, the method involves treating a subject having any disorder caused by proliferating stem cells. Such disorders, such as psoriasis, myelodysplasia, some autoimmune diseases, immuno-depression in aging, are treated by administering to the subject an effective amount of INPROL to partially inhibit proliferation of the stem cell in question.

BSPR:
The invention also includes a method of conducting ex vivo stem cell expansion [illegible] with INPROL and at least one

BSPR:
[illegible] INPROL

and (b) at least one inhibitory compound selected from the group consisting of MIP-1.alpha., TGF.beta., TNF.alpha., INF.alpha., INF.beta., INF.gamma., the pentapeptide pyroGlu-Glu-Asp-Cys-Lys, (SEQ ID NO;14) the tetrapeptide N-Acetyl-Ser-Asp-Lys-Pro, and the tripeptide glutathione (Gly-Cys-.gamma.Glu).

BSPR
The invention also includes a pharmaceutical composition comprising (a) INPROL and (b) at least one stimulatory compound selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-14, IL-15, G-CSF, GM-CSF, M-CSF, erythropoietin, thrombopoietin, stem cell factor, and flk2/flt3 ligand.

BSPR:
The current invention describes an inhibitor of stem cells (INPROL) which is different from those known in the art such as MIP-1.alpha., TGF.beta., the tetrapeptide of Frindel and colleagues or the pentapeptide of Paukovits and coworkers (cf., Wright & Pragnell, 1992 (op cit)). Naturally occuring INPROL has a molecular weight exceeding 10,000 daltons by ultrafiltration which distinguishes it from the tetrapeptide as well as the pentapeptide. It is more hydrophobic than MIP-1.alpha. or TGF.beta., in reverse phase chromatography systems, distinguishing it from those cytokines. Further, its mode of action is different from that of any previously described inhibitor in that it is active in an in vitro assay when used during a preincubation period only. MIP-1.alpha. for example, is not effective when used during a preincubation period only (Example 5). Further, naturally occuring INPROL is active in an assay measuring "high proliferative potential cells" (HPP-PFC) whereas MIP-1.alpha. is not (Example 6).

BSPV:
d) contacting said transfected hematopoietic cells ex vivo with INPROL,

BSPV:
f) optionally treating said mammal in vivo with INPROL.

DRPR:
FIG. 6 shows tritiated thymidine incorporation (cpm) into cells of the FDCP-mix line without (Control=0% Inhibition) and with various concentrations of INPROL purified from porcine bone marrow (pINPROL). Data are normalized against the control value.

DEPR:
INPROL reversibly inhibits division of stem cells. Specifically, INPROL is effective in temporarily inhibiting cell division of hematopoietic stem cells. Thus, the method of this invention may be employed in alleviating the undesirable side effects of chemotherapy on the patient's hematopoietic, myeloid and immune systems by protecting stem cells from damage caused by chemotherapeutic agents or radiation used to destroy cancer or virally infected cells. In one embodiment of the invention, INPROL is administered to the patient in a dosage sufficient to inhibit stem cell division while the chemotherapeutic agent acts on diseased cells. After the chemotherapeutic agent has performed its function, the stem cells inhibited by INPROL will, without further treatment, revert to dividing cells. If it is desired to enhance the regeneration of hematopoiesis, stimulatory growth factors or cytokines may be used in addition.

DEPR:
As used herein, the term "INPROL" includes mammalian proteins, purified as in the Examples, hemoglobin, the alpha chain of hemoglobin (with or without the heme group), the beta chain of hemoglobin (with or without the heme group), mixtures of alpha and beta chains (with or without the heme group), and

DEPR:

DEPR:
In another embodiment of the invention, pretreatment with INPROL allows for increased doses of chemotherapeutic agents or of radiation beyond doses normally tolerated in patients.

DEPR:
A large fraction of hematopoietic stem cells are normally quiescent (non-cycling). However, as a compensatory response to chemotherapy-induced hematopoietic damage, a larger proportion of stem cells enter into cycling after chemotherapy, which makes them particularly vulnerable to subsequent doses of cytotoxic chemotherapy or therapeutic irradiation. By inhibiting cycling of such stem cells, INPROL treatment permits earlier or more frequent administration of subsequent doses of cytotoxic chemotherapy, either at conventional or elevated doses.

DEPR:
In one embodiment of the invention, INPROL (0.1 mgs. to 6 gms - advantageously 1.0 to 60 mgs.) is administered about 24 hours to 10 days after an initial dose of chemotherapy. After another 4 to 60 hours, advantageously 24 to 48 hours, another dose of chemotherapy is administered. This cycle of alternating chemotherapy and INPROL is continued according to therapeutic benefit. Chemotherapy agents and protocols for administration are selected according to suitability for particular tumor types in standard clinical practice. Optionally, stimulatory growth factors such as G-CSF, stem cell factor, are used after chemotherapy or radiation treatment to further improve hematopoietic reconstitution.

DEPR:
For ex vivo applications 0.1 ng to 100 ng/10.sup.6 cells/ml, advantageously 20-50 ng/10.sup.6 cells/ml, of INPROL are used.

DEPR:
In another embodiment of the invention, INPROL is employed in a method for preparing autologous hematopoietic cells for transplantation. The hematopoietic cells are treated ex vivo with an effective amount of INPROL to inhibit stem cell division and then purged of cancerous cells by administering to the marrow cultures an effective amount of a chemotherapeutic agent or radiation. Chemotherapy agents with specificity for cycling cells are preferred. Marrow thus treated is reinjected into the autologous donor. Optionally, the patient is treated with an agent known to stimulate hematopoiesis to improve the hematopoietic reconstitution of the patient.

DEPR:
In another embodiment of the invention, INPROL is employed as an adjunctive therapy in the treatment of leukemia. For example, in disease states where the leukemic cells do not respond to INPROL, the leukemic hematopoietic cells cells are treated ex vivo with INPROL. The proliferation of normal stem cells is prevented by administration of INPROL. Thus, during the time that the proliferating leukemic cells are treated with a cell cycle-specific cytotoxic agent, a population of normal stem cells is protected from damage. Additionally, a stimulatory cytokine, such as IL-3 or GM-CSF, is optionally administered to induce cycling in the leukemic cells during drug or radiation treatment while the normal stem cells are protected with INPROL. The patient is treated with chemotherapy agents or radiation to destroy leukemic cells, and the purged marrow is then transplanted back into the patient to establish hematopoietic reconstitution.

DEPR:
Similarly, in another embodiment of the invention for treatment of patients with [illegible] viral infections that involve blood cells or lymphocytes, such as

DEPR:
In another embodiment of the invention, INPROL is employed to treat disorders

lotions, gels or patches) containing INPROL are employed where appropriate, as an alternative to parenteral administration. In most cases of leukemia, the leukemia progenitors are differentiated cell populations which are not affected by INPROL and which are therefore treated by methods using INPROL such as those described above. In cases where leukemia progenitors are very primitive and are directly sensitive to inhibition by INPROL, proliferation of leukemia cells is attenuated by administration of effective amounts of INPROL.

DEPR:
Antibodies, monoclonal or polyclonal, are developed by standard techniques to the INPROL polypeptides. These antibodies or INPROL polypeptides are labeled with detectable labels of which many types are known in the art. The labeled INPROL or anti-INPROL antibodies are then employed as stem cell markers to identify and isolate stem cells by administering them to a patient directly for diagnostic purposes. Alternatively, these labeled polypeptides or antibodies are employed ex vivo to identify stem cells in a hematopoietic cell preparation to enable their removal prior to purging neoplastic cells in the marrow. In a similar manner, such labeled polypeptides or antibodies are employed to isolate and identify epithelial or other stem cells. In addition, such antibodies, labeled or unlabeled, are used therapeutically through neutralization of INPROL activity or diagnostically through detection of circulating INPROL levels.

DEPR:
INPROL can be cloned from human gene or cDNA libraries for expression of recombinant human INPROL using standard techniques. For example, using sequence information obtained from the purified protein, oligonucleotide probes are constructed which can be labeled, e.g., with 32-phosphorus, and used to screen an appropriate cDNA library (e.g., from bone marrow). Alternatively, an expression library from an appropriate source (e.g., bone marrow) is screened for cDNA's coding for INPROL using antibody or using an appropriate functional assay (e.g., that described in Example 2). Hemoglobin itself, as well as the individual alpha and beta chains, have been cloned and expressed using methods known in the state of the art (see Pagnier et al., Rev. Fr. Transfus. Hemobiol. 35:407-15, 1992; Looker et al., Nature 356:258-60, 1992; Methods in Enzymology vol. 231, 1994). Each of these articles is hereby incorporated by reference.

DEPR:
In an advantageous embodiment, INPROL is the product of prokaryotic or eukaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. That is, in an advantageous embodiment, INPROL is "recombinant INPROL". The product of expression in typical yeast (e.g., Saccharomyces cerevisiae) or prokaryote (e.g., E. coli) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g.,non-human mammalian (e.g., COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, polypeptides of the invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention optionally also include an initial methionine amino acid residue (at position -1).

DEPR:
The present invention also embraces other products such as polypeptide analogs of the alpha, beta, gamma, delta, epsilon and/or zeta chain of hemoglobin. Such analogs include fragments of the alpha, beta, gamma, delta, epsilon and/or zeta chain of hemoglobin. Following well known procedures, one can readily design and manufacture genes coding for microbial expression of polypeptides having primary sequences which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternatively, modifications of cDNA and genomic genes can be readily accomplished by well-known site-directed

[redacted]

sequence or secondary conformations within the alpha, beta, gamma, delta, epsilon or zeta chains which fragments may possess one property of INPROL (e.g., receptor binding) and not others (e.g., stem cell inhibitory activity). It is noteworthy that activity is not necessary for any one or more of the products of the invention to have therapeutic utility (see, Weiland et al., Blut 44:173-5, 1982) or utility in other contexts, such as in assays of inhibitory factor antagonism. Competitive antagonists are useful in cases of overproduction of stem cell inhibitors or its receptor.

DEPR:
Homologous or analogous versions of INPROL from other species are employed in various veterinary uses, similar to the therapeutic embodiments of the invention described above.

DEPR:
INPROL acts on cycling stem cells by reversibly placing them in an undividing "resting" state. When it is desirable to stimulate the quiescent stem cells into division, e.g., after treatment of a patient with cancer chemotherapy agents or radiation, colony-stimulating factors and other hematopoietic stimulants are administered to the subject. Examples of such factors include but are not limited to: M-CSF (CSF-1), GM-CSF, G-CSF, Megakaryocyte-CSF, thrombopoieitin, stem cell factor or other cytokines, such as IL- 1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL- 13, IL- 14, or erythropoietin .

DEPR:
INPROL polypeptides or active fragments having stem cell inhibitory activity are purified or synthesized by conventional chemical processes combined with appropriate bioassays for stem cell inhibitory activity, as exemplified in the protocols described below.

DEPR:
In one embodiment of the invention, a therapeutically effective amount of the INPROL protein or a therapeutically effective fragment thereof is employed in admixture with a pharmaceutically acceptable carrier. This INPROL composition is generally administered by parenteral injection or infusion. Subcutaneous, intravenous, or intramuscular injection routes are selected according to therapeutic effect achieved.

DEPR:
Also comprehended by the invention are pharmaceutical compositions comprising therapeutically effective amounts of polypeptide products of the invention together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in INPROL therapy. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids, gels, ointments, or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent adsorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc. or into liposomes, niosomes, microemulsions, micelles, unilamellar or multilamellar vesicles, biodegradable injectable microcapsules or microspheres, or protein matrices, erythrocyte ghosts, spheroplasts, skin

[illegible] with [illegible] [illegible] [illegible] [illegible] INPROL [illegible] antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments [illegible] invention incorporate particular forms of

DEPR:
Following the subject's exposure to the cytotoxic agent or radiation, the therapeutic method of the present invention optionally employs administering to the subject one or more lymphokines, colony stimulating factors or other cytokines, hematopoietins, interleukins, or growth factors to generally stimulate the growth and division of the stem cells (and their descendants) inhibited by the prior treatment with INPROL. Such therapeutic agents which encourage hematopoiesis include IL1, IL2, IL-3, IL-4, IL-5, IL-6, IL-7, Meg-CSF, M-CSF (CSF-1), GM-CSF, G-CSF or erythropoietin. The dosages of these agents are selected according to knowledge obtained in their use in clinical trials for efficacy in promoting hematopoietic reconstitution after chemotherapy or hematopoietic stem cell transplant. These dosages would be adjusted to compensate for variations in the physical condition of the patient, and the amount and type of chemotherapeutic agent or radiation to which the subject was exposed. Progress of the reversal of the inhibition of the stem cells caused by administration of INPROL in the treated patient is monitored by conventional methods.

DEPR:
In the treatment of leukemia, it is beneficial to administer both INPROL to inhibit normal stem cell cycling and a stimulator of leukemic cell growth, such as IL-3 or GM-CSF, simultaneously with the cytotoxic drug treatment or during irradiation. By this protocol, it is possible to achieve the greatest differences between the cycling statuses and drug sensitivities of normal and leukemic cells.

DEPR:
The test data of INPROL presented in Table 1 demonstrate that cycling stem cells after treatment with INPROL become resistant to the action of .sup.3 H-Thymidine. For this and all of the following examples, the term "pINPROL" refers to the purified protein from porcine bone marrow. The same protection is seen for the S-phase specific cytotoxic drugs cytosine arabinoside and hydroxyurea (data not shown). If the treated stem cells are then washed with the cold media containing non-radioactive Thymidine, the surviving stem cells proliferate in mouse spleens to form colonies normally.

DEPR:
Using the following test system (Lord et al., in The Inhibitors of Hematopoiesis pp. 227-239, 1987) the direct effect of INPROL was shown. The multilineage factor (IL-3) dependent stem cell line, FDCP mix A4 (A4), was maintained in IMDM medium supplemented with 20% horse serum and 10% WEHI-3-conditioned medium as a source of colony-stimulating IL-3.

DEPR:
The studies of the effect of INPROL injected in vivo revealed that INPROL can effectively block the recruitment of CFU-S into cycle, thus protecting those cells from the cytotoxic effect of further treatment, showing its potential for clinical use.

DEPR:
The experimental protocol had two goals: to check the effect of INPROL on CFU-S when injected in vivo and to define the effective duration of INPROL activity in relation to cycling stem cells.

DEPR:
For the duration of the effect evaluation, one group of mice (21 mice per group) was injected with TSP only and another group was injected both with TSP and pINPROL (24 hours after TSP). The CFU-S cycling was measured every 24 hours during a week by taking 3 donors from each group and measuring CFU-S cycle status in their bone marrow by method described (see Example 1). Data presented

[illegible] chemotherapeutic agents like cytosine arabinoside or hydroxyurea are active in vivo. More importantly, for chemotherapeutic and radiation treatments having

DEPR:
The drug 5-fluorouracil (5-FU) drastically reduces the number of cells in the myeloid and lymphoid compartments. It is usually thought of as being cell-cycle specific, targeting rapidly proliferating cells, because incorporation of the nucleotide analogue into DNA during S-phase of the cell cycle or before results in cell death. The long-term survival and immunohematopoietic reconstitution of the bone marrow of mice is not affected by a single dose of 5-FU; however, it was demonstrated (Harrison et al. Blood 78:1237-1240, 1991) that pluripotent hematopoietic stem cells (PHSC) become vulnerable to a second dose of 5-FU for a brief period about 3-5 days after the initial dose. It can be explained that PHSC normally cycle too slowly for a single dose of 5-FU to be effective and are stimulated into rapid cycling by stimuli resulting from the initial 5-FU treatment. We have proposed that PHSC can be returned to a slow cycle status by INPROL and thus protected from the second 5-FU treatment.

DEPR:
FIG. 9 shows the increased survival after a single dose of pINPROL. The conditions of the model are clinically relevant for treating any cancer, including those characterized by solid tumors; such treatment would be administered to a patient having cancer by delivering an effective dose of INPROL between two consecutive dosages of radiation, thereby allowing greater dosages of radiation to be employed for treatment of the cancer. It should also be possible to extend this modality to chemotherapeutic agents.

DEPR:
Data are presented in FIG. 10. There was no cell growth seen in control cultures treated with AraC only, while in INPROL protected flasks regeneration of hematopoiesis occurred much more rapidly due to proliferation of progenitors from the adherent layer. Moreover, the cells from the experimental group when plated in agar grew only in the presence of IL-3 giving about 100 CFU per 50,000 cells; no leukemic cell growth was observed at least during 4 weeks. Thus, marrow treated ex vivo with an effective dose of AraC in combination with INPROL can be purged of cancerous cells while the stem cells are be protected. It should be possible to extend this modality to other forms of chemotherapy or radiation treatments.

DEPR:
The increase of MRA induced by preincubation with INPROL could be one of the mechanisms in the improving of the radioprotection. To examine this hypothesis, MRA was measured according to Visser et al. (op. cit.). Briefly, the donor BDF1 mice were pretreated with testosterone, their bone marrow was preincubated with medium or pINPROL for 4 hours and injected into irradiated animals. On Day 13, the bone marrow cells from recipient femurs were plated in agar in 3 different concentration (0.01, 0.05, 0.1 equivalent of a femur) in the presence of 20% of horse serum and 10% of WEHI-CM. The number of Day 7 colonies represented the MRA as far as the colony-forming cells in the bone marrow of recipients at the time were the progenitors of the donor's immature stem cells.

DEPR:
As can be seen on FIG. 12 the MRA of the preincubated with INPROL cell population is greater than in the control group (B).

DEPR:
As is obvious from Table 4, the incubation of normal bone marrow (NBM) from intact young animals (BDF1 8-12 weeks old) with INPROL did not change the number or proportion of different types of colonies. BDF.sub.1 donors pretreated with Testosterone Propionate (TSP) showed the same increase in CFU-S proliferation as was seen before (Example 1, 3, 4) a slight increase in the erythroid progenitor number (BFU-E colonies) and a decrease in GM-CFU, which

[illegible] pretreated with TSP and in older (24-25 month old) BDF1, which have in common the abnormally high level of CFU-S proliferation. The correction of both the proliferation of CFU-S and the differentiation was induced by the incubation

It has been observed that the incubation of bone marrow cells containing a high proportion of proliferating CFU-S with INPROL not only changes the cycling of CFU-S, but also their differentiation, switching the predominantly erythroid differentiation in favor of granulocytic and lymphoid progenitors. This property of INPROL is of importance due to the immunosuppression side effects of cytotoxic chemotherapy or radiotherapy, as well as the immunosuppression accompanying hyperproliferative stem cell disorders and aging.

DEPR:
The example shows the direct effect of INPROL on the differentiation of immature precursors from the Lymphoid Long Term Culture (LLTC) established according to Wittlock & Witte (Ann. Rev. Immun. 3:213-35, 1985) into pre-B progenitors, measured by the formation of colonies in methylcellulose containing IL-7.

DEPR:
LLTC were established as described and fed with fresh LLTC-media (Terry Fox Labs., Vancouver, Canada) twice a week. Nonadherent cells were harvested once a week, washed free of factors and incubated for 4 hours with 25 ng/ml pINPROL or medium alone for control. After the incubation, the cells were washed and plated at a concentration of 10.sup.5 cells/ml in methylcellulose, containing 30% FCS, and 10 ng/ml of IL-7. Data from 3 weeks are shown in FIG. 13. The number of large pre-B colonies varied in control, increasing with time, but preincubation with INPROL always stimulated the growth of colonies 4 to 8 fold above the control level. This demonstrates an immunostimulatory property of INPROL which is of use in correcting immunodeficient states and in increasing desired immune responses, e.g., to vaccination.

DEPR:
The following procedure was used to examine the effect of INPROL on the number of clonogenic cells (LTC-IC) among bone marrow transplant cells established from the peripheral blood of a patient with CML.

DEPR:
Data presented on FIG. 14 show that there was no loss in LTC-IC during the first 10 days of culture initiated from the healthy donor's bone marrow and approximately 30% of the number of input LTC-IC were still present after 5 weeks in culture. The number of the CML patient's LTC-IC was drastically reduced to about 8% during the 10 day period and did not recover during further incubation, while the preincubation of cells with INPROL increased the LTC-IC level to 30% of initial number and it was maintained during 8 weeks.

DEPR:
Clinically relevant applications of INPROL predicted by these preliminary data include their use in strategies for selectively improving the normal stem cell content of fresh or cultured marrow transplants, strategies for enhancing the recruitment of residual normal stem cells in vivo also protocols for transferring new genetic material into human marrow stem cells for the further transplantation into patients.

DEPR:
An analysis of the material obtained by collecting only the second major HPLC peak is shown in FIG. 15 (A and C). Material containing both peaks (e.g., FIG. 5) will be referred to herein as pINPROL Preparation 1 and those consisting of only the second peak will be referred to as pINPROL Preparation 2. 500 ug of this active, purified pINPROL Preparation 2 was loaded onto a C4 reverse phase column (Vydac) and eluted using a linear gradient of 595% acetonitrile in 0.1% trifluoroacetic acid. The material eluted as a single peak at 53% acetonitrile (FIG. 15A). When 250 .mu.g of MIP-1.alpha. (R&D Systems), however, was run under identical conditions, it eluted at 43.9% acetonitrile (FIG. 15B--note

DEPR:
To confirm that hemoglobin beta chain has INPROL activity, a suicide assay

contrast, cells from testosterone-treated animals incubated with either pINPROL or purified hemoglobin beta chain at 40 ng/ml showed a dramatic lowering of the percentage of cells in cycle from 36% to 0% and to 7%, respectively. The higher dose of 200 ng was less effective for both proteins. As a positive control, the previously characterized stem cell inhibitor MIP-1.alpha. reduced cycling to 13%.

DEPR:
The foregoing provides evidence that the beta chain of porcine hemoglobin exhibits INPROL activity. Other data (e.g., Table 9, FIG. 20) demonstrate that isolated alpha chain, as well as intact hemoglobin, are also active as stem cell inhibitors. Active preparations also include mixtures of alpha and beta chains (e.g., FIG. 5).

DEPR:
In order to investigate the ability of purified INPROL from porcine bone marrow to affect cycling on human progenitors, umbilical cord blood cells were obtained. Either the total mononuclear cell fraction obtained after separation on Ficoll or the CD34+ fraction obtained after fractionation on anti-CD34 affinity columns (CellPro Inc.) was used. Cells were incubated for 48 hours in vitro in the presence of interleukin 3 (IL-3) and stem cell factor (SCF) (100 ng/ml each) in order to ensure that the early stem cells were in cycle. After this preincubation, cycling assays were conducted as described in Example 14 for the mouse except that CFU-GEMM (instead of CFU-MIX) were counted on Day 18 after plating. As shown in Table 15, porcine INPROL inhibited cycling of CFU-GEMM in either the bulk mononuclear cells or in the CD34+ fraction.

DEPR:
Human umbilical cord blood mononuclear cells were obtained and incubated in IL-3 and SCF and used in a cycling assay as described in Example 18. As shown in Table 16, both porcine INPROL purified from bone marrow and human alpha hemoglobin, purified from peripheral blood, were active in this assay.

DEPL:
1. Long-term Bone Marrow Culture L1210 Leukemia Model For The Study Of INPROL Effect Preserving Normal Hematopoiesis During Purging With AraC

DEPL:
2. Marrow Repopulating Ability (MRA) And Thirty Days Radioprotection Are Increased By INPROL Treatment In Vitro

DEPC:
Inhibition of CFU-S Proliferation by INPROL Injected in vivo: Doses and the Duration of the Effect

DEPC:
Most Primitive Hematopoietic Stem Cells Stimulated to Cycle Rapidly After Treatment with 5-FU are Protected by INPROL from the Second 5-FU Exposure

DEPC:
Effects of Pre-Incubation with INPROL vs. MIP-1.alpha. in Bone Marrow Cells

DEPC:
INPROL inhibits HPP-CFC proliferation

DEPC:
INPROL Therapy Effect on the Recovery from Radiation-induced Bone Marrow Aplasia

DEPC:

DEPC:
Immunostimulatory Activity of INPROL

Alternative method for isolating larger quantities of INPROL

DEPC:
Active INPROL Preparations Contain Hemoglobin Beta Chain

DEPC
Active INPROL Preparations Contain Hemoglobin Alpha Chain

DEPC:
Further characterizations of Porcine INPROL

DEPC.
Purified INPROL, Purified Porcine Alpha Hemoglobin or Purified Porcine Beta Hemoglobin are Active In Vivo

DEPC:
Porcine INPROL is Active on Human Mononuclear or CD34+ Cord Blood Cells In Vitro

DETL:
TABLE 4 _____ INPROL Effect On CFU-S Differentiation Into Committed Progenitors BFU-E and CFU-GM Percent Donors Of CFU-S Bone Killed by Marrow pINPROL .sup.3 HTdR BFU-E CFU-GM _____ BDF.sub.1 Young - 12.0 .+-. 0.3 28.33 .+-. 1.91 46.22 .+-. 3.44 + 15.0 .+-. 1.3 22.00 .+-. 3.74 47.70 .+-. 3.72 BDF.sub.1 Old - 47.1 .+-. 1.9 43.75 .+-. 1.54 24.0 .+-. 1.33 + 11.4 .+-. 0.7 15.25 .+-. 1.45 44.0 .+-. 7.63 BDF.sub.1 - 53.2 .+-. 1.6 32.67 .+-. 2.44 15.71 .+-. 2.28 Stimulated + 7.2 .+-. 0.4 12.00 .+-. 1.83 35.50 .+-. 1.4 by TSP Balb/C - 57.0 .+-. 1.9 47.60 .+-. 2.96 33.57 .+-. 3.45 + 23.0 .+-. 2.4 24.86 .+-. 2.53 70.60 .+-. 4.96 _____

Document Number 1

Entry 1 of 4 File: USPT Aug 17, 1999

US-PAT-NO: 5939391
DOCUMENT-IDENTIFIER: US 5939391 A

TITLE: Hemoglobin alpha chain peptide fragments useful for inhibiting stem cell proliferation
DATE-ISSUED: August 17, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsyrlova; Irena	Gaithersburg	MD	N/A	N/A
Wolpe; Stephen D.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 514/14; 530/326, 530/327, 530/385

CLAIMS:

What is claimed is:
1. A peptide having the sequence
Phe-Pro-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val (SEQ ID NO:1).
2. A cyclic peptide having the sequence
Cys-Phe-Pro-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val-Cys (SEQ ID NO:2) where the two Cys residues form a disulfide bond.
3. A pharmaceutical composition comprising the peptide of claim 1.
4. A pharmaceutical composition comprising the peptide of claim 1 in unit dosage form.
5. A pharmaceutical composition comprising the peptide of claim 1, wherein the peptide concentration in said composition is 1 to 100 ng/ml.
6. A pharmaceutical composition comprising the peptide of claim 2.
7. A pharmaceutical composition comprising the peptide of claim 2 in unit dosage form.

Document Number 1

Entry 1 of 2 File: USPT Aug 17, 1999

DOCUMENT-IDENTIFIER: US 5939391 A
TITLE: Hemoglobin alpha chain peptide fragments useful for inhibiting stem cell proliferation

BSPV:
Phe-Pro-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val (SEQ ID NO:1),

DEPV:
43-55 Phe-Pro-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val (SEQ ID NO:1)

CLPR:
1. A peptide having the sequence
Phe-Pro-His-Phe-Asp-Leu-Ser-His-Gly-Ser-Ala-Gln-Val (SEQ ID NO:1).

Search Results - Record(s) 1 through 2 of 2 returned.

1. Document ID: US 5939391 A

Entry 1 of 2 File: USPT Aug 17, 1999

US-PAT-NO: 5939391
DOCUMENT-IDENTIFIER: US 5939391 A

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Tsyrlova; Irena	Gaithersburg	MD	N/A	N/A
Wolpe; Stephen D.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 514/14; 530/326, 530/327, 530/385

ABSTRACT:

Disclosed and claimed are methods for the isolation and use of stem cell inhibiting factors for regulating the abnormal stem cell cycle and for accelerating the post-chemotherapy peripheral blood cell recovery. Also disclosed and claimed are the inhibitors of stem cell proliferation.
7 Claims, 28 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 23

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Image

2. Document ID: US 5861483 A

Entry 2 of 2 File: USPT Jan 19, 1999

US-PAT-NO: 5861483
DOCUMENT-IDENTIFIER: US 5861483 A

TITLE: Inhibitor of stem cell proliferation and uses thereof

DATE-ISSUED: January 19, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wolpe; Stephen D.	Rockville	MD	N/A	N/A

US-CL-CURRENT: 530/385; 435/69.1, 530/350, 530/380

ABSTRACT:

The present invention provides polypeptides and compositions containing same which include a hemoglobin alpha chain wherein the C-terminal hydrophobic domain has been modified.
15 Claims, 31 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 27

Term	Documents
PHE-PRO-HIS-PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL	2
PHE-PRO-HIS-PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VALS	0
FPHPDLSHGSAQV	0
FPHPDLSHGSAQVS	0
FPHPDLSHGSAQV OR PHE-PRO-HIS-PHE-ASP-LEU-SER-HIS-GLY-SER-ALA-GLN-VAL	2